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SO CLOSE AND YET SO FAR – MOLECULAR MICROBIOLOGY OF *CAMPYLOBACTER FETUS* SUBSPECIES

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Campylobacter fetus comprises two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*, which are considered emerging pathogens in humans and animals. Comparisons at the genome level have revealed modest subspecies-specific variation; nevertheless, these two subspecies show distinct host and niche preferences. *C. fetus* subsp. *fetus* is a commensal and pathogen of domesticated animals that can be transmitted to humans via contaminated food. The clinical features of human infection can be severe, especially in impaired hosts. In contrast, *C. fetus* subsp. *venerealis* is a sexually transmitted pathogen essentially restricted to cattle. Infections leading to bovine venereal campylobacteriosis cause substantial economic losses due to abortion and infertility. Recent genome sequencing of the two subspecies has advanced our understanding of *C. fetus* adaptations through comparative genomics and the identification of subspecies-specific gene regions predicted to be involved in pathogenesis. The most striking difference between the subspecies is the highly subspecies-specific association of a pathogenicity island in the *C. fetus* subsp. *venerealis* chromosome. The inserted region encodes a Type 4 secretion system, which contributes to virulence properties of this organism *in vitro*. This review describes the main differences in epidemiological, phenotypic, and molecular characteristics of the two subspecies and summarizes recent advances towards understanding the molecular mechanisms of *C. fetus* pathogenesis.

Keywords: *Campylobacter fetus* subspecies, human/animal pathogens, comparative genomics, pathogenicity

Introduction

Campylobacters are zoonotic bacterial pathogens causing human and animal disease. The most prominent member of this genus is *C. jejuni*, which is the main cause of human bacterial diarrhea. *C. jejuni* and *C. coli* account for over 95% of Campylobacters isolated in cases of human infections [1–3]. Nevertheless, *C. fetus* is recognized as a relevant pathogen of livestock, highly adapted to the mucosa of the intestinal and/or urogenital tract of its hosts. *C. fetus* is also a human pathogen. It is the *Campylobacter* species most often isolated from human blood in cases of bacteremia, and the number of reported *C. fetus* infections is believed to be substantially underestimated [2, 4]. *C. fetus* comprises two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*, which display strikingly different host and niche preferences, although they are highly related at the genome level.

Epidemiology and clinical significance

Although the two subspecies *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* share many characteristics, they display a different microbiology, epidemiology and features of infection in humans (*C. fetus* subsp. *fetus*) and livestock (*C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*) [5, 6], as summarized in *Table 1*.

C. fetus subsp. *fetus* is the predominant subspecies infecting humans [6]. The natural habitat of *C. fetus* subsp. *fetus* is the intestine of sheep and cattle, where it is considered to be a commensal. Additionally, it can be isolated from swine, poultry, and reptiles [2, 7–9]. *C. fetus* subsp. *fetus* is considered as an opportunistic pathogen causing severe diseases, particularly in the elderly and immunocompromised patients [4]. Transmission is believed to occur primarily by ingestion of contaminated food or water, although an outbreak of *C. fetus* diarrhea in Alberta, Canada, was associated with working in the community slaughterhouse [10]. The first human *C. fetus* infection was reported in 1947, associated with the consumption of unpasteurized milk [4]. Typically, patients with *C. fetus* subsp. *fetus* bacteremia report consuming raw milk, raw beef, or beef liver, or undercooked pork [11–13]. *C. fetus* subsp. *fetus* colonizes the intestinal tract, which can cause acute diarrhea. More importantly, primary infection leads to portal bacteremia, and *C. fetus* subsp. *fetus* colonizes

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subsequently the liver. Excretion via the biliary tract leads to a second phase of intestinal colonization. Healthy individuals usually eliminate *C. fetus* subsp. *fetus* and there is either transient or no systemic bacteremia [4]. In impaired hosts, especially the elderly, infants, or patients with an underlying illness such as alcoholism, diabetes, HIV infection, or cancer, severe *C. fetus* subsp. *fetus* infection can occur [12, 14–18]. In these cases, infection results in septicemia, peritonitis, endo- and pericarditis, cellulitis, meningoencephalitis, and osteoarthritis. Interestingly, *C. fetus* subsp. *fetus* is often isolated from infected vessels (e.g. infected arterial aneurysms). Patients often experience relapsing infections, which is at least partly conferred by antigenic variation of the pathogen (for details see section "Pathogenesis of *C. fetus* infection"). Human abortions resulting from infection occur rarely [13, 19–22]. It is notable, however, that *C. fetus* subsp. *fetus* exhibits pathogenicity in animals, causing bovine, ovine, and caprine abortion. A fecal–oral route of transmission is described, and the course of infection is not considered epidemic [5].

C. fetus subsp. *venerealis* is predominantly a bovine pathogen. It colonizes the urogenital tract of cattle [5]. The reservoir of *C. fetus* subsp. *venerealis* are the epithelial crypts of the prepuce. This infection is often asymptomatic but persists throughout the animal's lifetime [23]. Cows are infected during coitus, wherein *C. fetus* subsp. *venerealis* colonizes the vagina, cervix, uterus, and oviducts, leading to an ascending infection causing infertility and abortion. Despite an immune response, *C. fetus* subsp. *venerealis* can persist in the bovine vagina. The persistence in the prepuce and vagina is the major factor for the epidemic spread of *C. fetus* subsp. *venerealis* leading to bovine venereal campylobacteriosis (BVC). BVC is a statutory disease with worldwide distribution [5]. It is important for cattle breeding and the international trade of embryos, and it places a significant economic burden on the artificial insemination industry [24].

Differentiation, isolation, and typing of *C. fetus* subspecies

Phylogeny and subspeciation

C. fetus can be either serotypes A, B, or AB based on its different O-antigens (i.e. LPS). *C. fetus* subsp. *venerealis* is always type A, whereas *C. fetus* subsp. *fetus* might be type A, type B, or rarely type AB [25–27]. The different serotypes correspond also to the different S-layer protein types. Since *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus* are both LPS/sap type A, differentiation between subspecies likely occurred after the type A–type B split [28]. Multilocus sequence typing (MLST) studies have also emphasized that *C. fetus* subsp. *venerealis* represents a "bovine" clone of *C. fetus* subsp. *fetus* type A [29]. Interestingly, comparison of 16S rRNA sequences of *C. fetus* strains originating from mammals and reptiles revealed a phylogenetic clustering of the reptile strains between mammalian *C. fetus* subspecies and *C. hyointestinalis* [28]. Furthermore, the *C. fetus* reptilian and mammalian strains show higher genetic divergence than between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* and between type A and type B strains [30]. Detailed genome analysis of *C. fetus* strains from reptiles support the definition of a new *C. fetus* reptile subspecies [31, 32]. *Figure 1* describes the proposed ancestral relationship between *C. fetus* subspecies, based on the phylogenetic studies mentioned above.

Isolation, identification and subspecies differentiation

Collection of specimens, the choice of transport and isolation media, as well as the use of selective techniques such as filters all affect *C. fetus* isolation rates (reviewed in Ref. [33]). The slow growth characteristics of *C. fetus* limit detection compared to the other nonfastidious bacteria present in the specimens [2, 34]. Cephalothin-containing selective media inhibit the growth of *C. fetus* [4, 6, 35, 36]. Altogether, this contributes to a pronounced underestimation of *C. fetus* infections.

Differentiation between *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* is difficult, although mandatory, since only *C. fetus* subsp. *venerealis* represents the agent of the statutory disease BVC [24, 33]. Several assays for phenotypic and genotypic differentiation have been established [33]. The currently recommended gold standard in differentiation is the 1% glycine tolerance test, wherein *C. fetus* subsp. *fetus* is glycine-tolerant [37]. Biochemical assays based on H_2S production and selenite reduction or aerotolerance and growth at 42 °C are reported to distinguish between the subspecies but often yield inconsistent results [33]. A distinct group of *C. fetus* strains designated as *C. fetus* subsp. *venerealis* biovar intermedius was described, which react positively in the H_2S test (typically positive for *C. fetus* subsp. *fetus*) but otherwise resembled

Table 1. *C. fetus* subspecies specific host and tissue preferences and underlying diseases

Subspecies	Source	Tissue	Human disease	Animal disease
C. fetus fetus	Cattle, sheep, reptiles	Intestinal tract, urogenital tract	Septicemia, endo-, pericarditis, peritonitis, meningoencephalitis, cellulitis, arthritis, abortion	Abortion
C. fetus venerealis	Cattle	Urogenital tract	Septicemia (uncommonly)	BVC, Infertility, abortion

C. fetus subsp. *venerealis* [38]. Also, MLST data showed that these strains had the same sequence type as *C. fetus* subsp. *venerealis* [29].

The need for more reliable assays led to the application of molecular methods for subspecies discrimination. One of the first polymerase chain reaction (PCR) based analysis methods developed is the multiplex PCR described by Hum et al. [39]. Unfortunately, this method cannot reliably identify biovar intermedius strains [29]. Further PCR assays were described, modifying the original PCR tools or applying new subspecies-specific primers [40–44]. The first reliable molecular method for differentiation was the amplified fragment length polymorphism (AFLP) fingerprinting described by Wagenaar et al. [45], which also supports discrimination of biovar intermedius [29]. MLST confirmed PCR and AFLP data [29]. Evaluation of glycine tolerance in combination with these molecular methods is sufficient and reliable to distinguish *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, and *C. fetus* subsp. *venerealis* biovar intermedius [33].

Genomics of *C. fetus* subspecies

Comparative analyses

Genome comparison is an indispensable tool for understanding bacterial biology and virulence. The first available complete *Campylobacter* genome was from the clinical *C. jejuni* isolate NCTC 11168 [46]. It has a circular, highly dense, and rather small genome (~1.6 Mb) with a low G + C content (~30%). Surprisingly, this *C. jejuni* genome lacks any insertion elements or prophages [46]. Early pulsed-field gel electrophoresis studies revealed a smaller genome size for *C. fetus* subsp. *fetus* (~1.2 Mb) and *C. fetus* subsp. *venerealis* (1.3–1.5 Mb), although variations between different isolates have been reported [47]. In 2006, the complete genome sequence of *C. fetus* subsp. *fetus* strain 82-40, a human blood isolate, was published (GenBank acc. no. NC_008599) with a genome size of about 1.8 Mb and more than 90% coding sequence. Recently, the unfinished genome sequences of an Argentinean cattle isolate *C. fetus* subsp. *venerealis* strain AZUL-94 [48] and the *C. fetus* subsp. *venerealis* type strain NCTC 10354^T [49] were published and revealed a genome size of ~1.9 Mb and a G + C content of ~33%. Alignment of the sequences revealed that the genomes of *C. fetus* subspecies are highly syntenic with more than 99% average amino acid identity between the core proteomes [48, 50]. Interestingly, comparison between the core proteomes of reptilian and mammalian *C. fetus* subsp. *fetus* revealed a significant lower level of amino acid identity (~94%), underscoring that reptilian *C. fetus* represents a separate subspecies [32].

Representational difference analysis (RDA) and a complete genome sequencing project of the *C. fetus* subsp. *venerealis* strain 84-112 [25] in our laboratory helped to identify gene regions uniquely present in one of the two *C. fetus* subspecies and to understand their different lifestyles

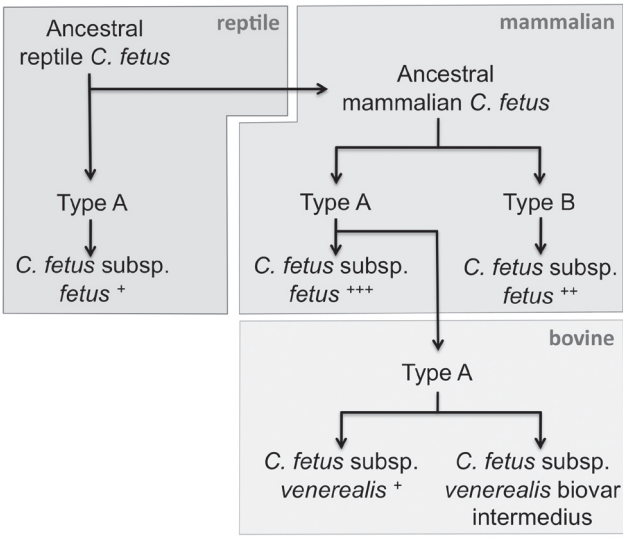

Fig. 1. Proposed evolutionary relationships of *C. fetus* subspecies. 16S rRNA analysis, MLST data, and DNA hybridizations suggest that *C. fetus* subsp. originate from an ancestral reptile strain. *C. fetus* subsp. *fetus* serotypes A and B correspond to the two different surface array protein (sap) types A and B expressed in strains adapted to mammals. *C. fetus* subsp. *venerealis* (exclusively type A) is considered a bovine clone evolved from subsp. *fetus*. Reptile *C. fetus* subsp. *fetus* are type A and are genetically more diverse than mammalian *C. fetus* subsp. *fetus* types A and B. It is proposed, therefore, that they represent a distinct, still unclassified subspecies (adapted from Ref. [30]). The frequency at which the respective subspecies cause human infection is indicated with plus marks (+ to +++).

[51, 52]. Despite the high homology between the subspecies, a striking number of interesting genes were identified that might contribute to the differences in host specificity and adaption (manuscript in preparation [53]) including a *C. fetus* subsp. *venerealis*-specific genomic pathogenicity island (PAI) (see below), which was absent from all the tested *C. fetus* subsp. *fetus* strains [51]. Conversely, a region encoding for a homolog of GDP-mannose 4,6-dehydratase (GMD) was identified uniquely in *C. fetus* subsp. *fetus* [51]. This enzyme is involved in GDP-*L*-fucose synthesis needed for LPS synthesis [54, 55]. In *Helicobacter pylori*, mutation of GMD leads to a reduced acid resistance and an altered LPS structure [56]. Given that *C. fetus* subsp. *fetus* primarily colonizes the intestine, it must first survive passage through the acidic gastric environment, providing a reasonable explanation for this finding.

Pathogenicity island of C. fetus *subsp.* venerealis

The major difference between the two *C. fetus* subspecies is the presence of a PAI of about 45 kb nearly exclusively found in *C. fetus* subsp. *venerealis*. The PAI harbors homologs of genes required for the assembly of a Type 4 secretion system (T4SS) [48, 51]. The open reading frames (ORFs) *orf7* to *orf18* of the PAI are related to genes for the secretion components VirB2 to VirB11 and VirD4 dis-

playing highest homologies to the mobilizable tetracycline resistance plasmids pCC31 of *C. coli* and pTet of *C. jejuni*. These plasmids are supposed to play an important role in the plasticity of *Campylobacter* genomes [51, 57, 58]. In addition, mobility genes such as a bacteriophage P4-like integrase and two homologs to the *H. pylori* transposable element IS*Hp608* [59] are present in the PAI, marking the island as part of the horizontal gene pool [60]. A methionyl-tRNA at the 5´ end of the PAI and tRNA remnants at the 3´ boundary indicate the sites of integration into the backbone genome *C. fetus* subsp. *venerealis*. Two putative effector proteins called Fic1 and Fic2 (*orf38* and *orf39*), which may be secreted by the T4SS to mammalian hosts, were identified downstream in close proximity to the T4S locus [51]. Initial Southern blot and PCR analyses revealed that the PAI is exclusively present in *C. fetus* subsp. *venerealis* [51]. Recently, a *C. fetus* subsp. *fetus* strain isolated from a calf in Switzerland was reported to harbor the PAI. In this strain, resistance genes for tetracycline [*tet(44)*] and streptomycin [*ant(6)-Ib*] are inserted into the PAI, suggesting that, under certain selective pressures, the PAI can be transferred between the subspecies and stably maintained [61].

Type 4 secretion and effector proteins

Bacterial pathogens use T4SS, which are membrane-associated multiprotein complexes, to deliver effector proteins and DNA to host cells [62–65]. The T4SS of *C. jejuni*, located on plasmid pVir [66, 67], mediates *in vitro* adherence and invasion. Mutation of *virB11* led to reduced development of diarrhea in the ferret diarrhea model [67]. The *C. fetus* subsp. *venerealis* T4SS is encoded on a PAI, which is nearly exclusively present in this subspecies. It is thus reasonable to propose that this virulence gene cluster contributes to the niche preference of this subspecies [48, 51]. When HeLa cells were infected with *C. fetus* subsp. *venerealis* wild-type and the isogenic *virB9* mutant, diminished killing of eukaryotic cells was observed compared to the wild-type strain [51]. Moreover, a *virD4* mutant showed reduced invasion levels of Caco-2 cells compared to the wild type, demonstrating the involvement of the *C. fetus* T4SS in host cell invasion [51]. A recombinant plasmid carrying heterologous mobility features from a *C. coli* plasmid could be transferred from *C. fetus* subsp. *venerealis* to *C. fetus* subsp. *fetus* and *E. coli*, suggesting that the T4SS is proficient in plasmid DNA transfer to other bacteria. Inactivation of *virB9* and *virD4* of the T4SS reduced interbacterial plasmid transfer significantly [68].

Downstream of the VirB/VirD4 locus, two ORFs have been identified, which harbor conserved core motifs of the FIC (*f*ilamentation *i*nduced by *c*AMP) superfamily [51, 68]. Proteins carrying the FIC domains (HPFxxGNGR) are present in both bacteria and eukaryotes and play a role in cell division and cell signaling [69, 70]. Importantly, FIC domain-containing proteins are known to be bacterial effectors of *Vibrio parahaemolyticus*, *Legionella pneumophila,* and *Bartonella henselae* which are transported into the host cells via Type 3 or Type 4 secretion. In host cells, the bacterial FIC domain-containing proteins target cellular GTPases. The interactions result in cytoskeletal rearrangement or fragmentation of the Golgi apparatus, finally leading to host cell apoptosis or necrosis. By contrast, bacterial FIC proteins, such as those of *B. henselae,* can also act to prevent the host cells from apoptosis to promote bacterial persistence [71–74]. The immediate downstream localization of the *C. fetus* subsp. *venerealis fic1* and *fic2* genes to the VirB/VirD4 locus, together with the positively charged secretion motif at the C-terminus of the predicted proteins, make them likely candidates for Type 4 secretion. To assess whether these Fic proteins might be translocated via the T4SS, CRAfT assays [75] were performed between bacteria, which indicated a secretion at least of Fic2 [68]. *In vitro* studies including a translocation reporter assay with cultured human cells are necessary to clarify whether *C. fetus* subsp. *venerealis* translocates Fic to host cells via the Type 4 secretion apparatus and ultimately to define their pathogenic properties [76].

Pathogenesis of *C. fetus* infection

The virulence mechanisms of the two *C. fetus* subspecies and the resulting host and niche preferences remain poorly understood. The lack of suitable animal models for *C. fetus* infection has hampered extensive *in vivo* studies; therefore knowledge about *C. fetus* pathogenicity is mainly derived from *in vitro* cell-culture-based infection assays. The following section describes virulence factors employed by both *C. fetus* subspecies. *Figure 2* summarizes the possible infection mechanisms *C. fetus* subspecies utilize to infect their hosts.

Attachment and invasion

Attachment of bacterial pathogens to epithelial cells is a prerequisite for invasion of host cells and subsequent translocation to deeper layers of the mucosa. The spiral cell shape and the corkscrew-like motility conferred by the flagella of *C. fetus* are necessary to colonize and cross the mucus barrier overlaying the epithelium. Flagella mutants of *C. jejuni* are highly attenuated in virulence. Moreover, the flagellum has been shown to be an important adhesin for *C. jejuni* and may have a similar function in *C. fetus* [77, 78]. The genomes of both *C. fetus* subspecies harbor homologs of the adhesin PEB1, which is an outer membrane protein, and CadF. CadF is a fibronectin-binding protein that mediates *Campylobacter* binding to the extracellular matrix of the epithelium and appears to be essential for cellular uptake preferentially at the basolateral cell surface [79]. *C. fetus* has been shown to adhere to and invade human epithelial cells such as INT 407 and Caco-2 [51, 80, 81]. Gentamicin protection assays revealed also intracellular replication of *C. fetus* in INT 407

cells, although the intracellular niche of *C. fetus* has not been defined up to now [80]. Binding of *C. fetus* to immobilized fibronectin and enhancement of INT 407 cell invasion by fibronectin have been shown [82]. Host cell invasion by *C. jejuni* was reported to occur via two different processes. One is microtubule-dependent and occurs mainly on the apical cell surface. The second mechanism is microfilament-dependent and is observed on the basolateral side of the cells, presumably contributing to reinfection of cells after *C. jejuni* translocation across the epithelium [77]. Using polarized Caco-2 cells as a model for the gut epithelium, Baker and Graham observed that *C. fetus* crosses the epithelium without disturbing the integrity of the monolayer. These authors therefore proposed that *C. fetus* prefers the transcellular route to pass the epithelial barrier [83]. Moreover, the authors reported that *C. fetus* invasion and translocation across Caco-2 cells occurs in an actin-independent manner, whereas a functional tubulin cytoskeleton is required for translocation but not for invasion of Caco-2 cells [83]. This finding is contrary to the situation with *C. jejuni* and other Campylobacters, such as *C. concisus,* which translocate across a cell monolayer via the paracellular route, induce epithelial barrier dysfunction, and ultimately initiate epithelial cell apoptosis and necrosis [84, 85]. Migration of bacteria underneath the host cells prior to efficient invasion, a process termed subvasion, was reported for *C. jejuni* [86]. Confocal microscopy of *C. fetus*-infected Caco-2 monolayers displayed also migration of the bacteria underneath the eukaryotic cells [52]. Moreover, transwell gentamicin protection assays exhibited lower invasion levels of *C. fetus* and *C. jejuni* compared to standard assays if gentamicin was applied to the basolateral compartment, suggesting a similar subvasion mechanism employed by *C. fetus* [52].

Cytolethal distending toxin and other virulence factors

Campylobacter species produce the cytolethal distending toxin Cdt, consisting of three subunits encoded by *cdtA*, *cdtB* and *cdtC* [87]. All three subunits are required for full toxicity, forming the active enzyme complex, which is bound to the host cell membrane via CdtA and CdtC [88, 89]. CdtB shows DNase I-like activity causing DNA damage and leads to a cell cycle arrest in G2 phase and host cell enlargement [90]. The proposed involvement of Cdt in

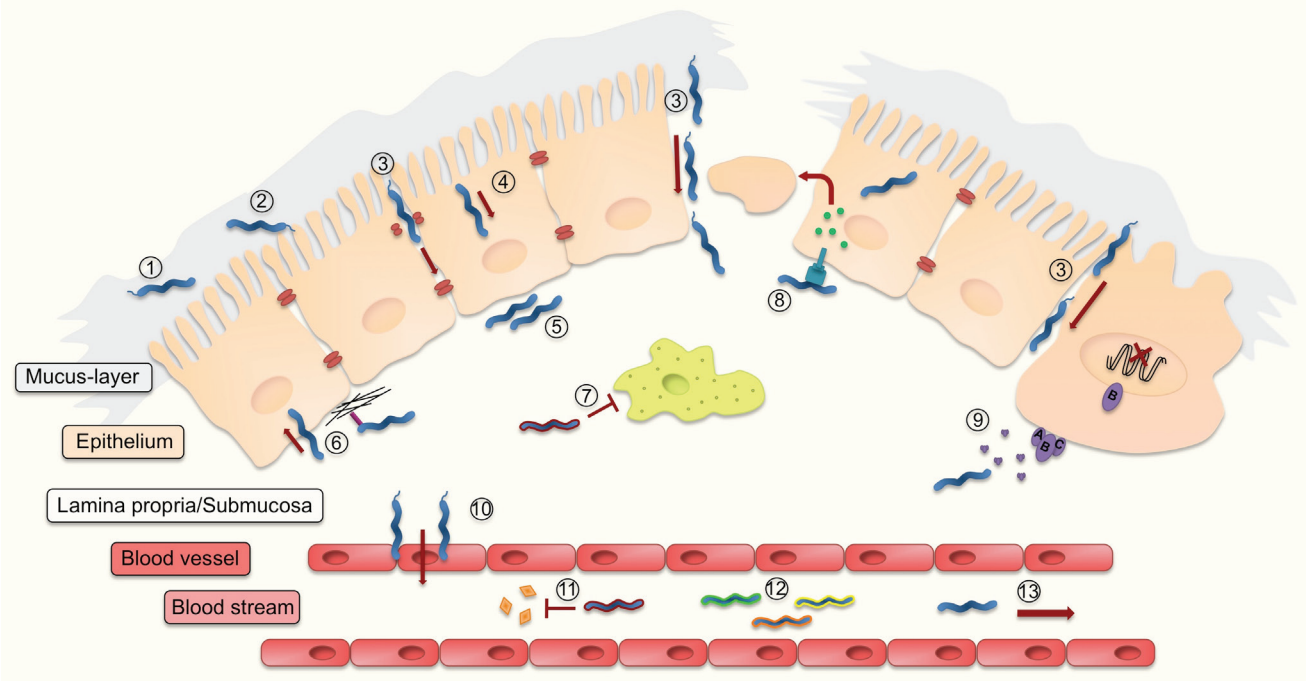

Fig. 2. Model for *C. fetus*' interaction with epithelial cells and proposed mechanisms of infection. (1) *C. fetus* is highly adapted to mucosal surfaces owing to its spiral shape and the flagellum-based corkscrew-like motility. (2) Adherence to epithelial cells occurs via the flagellum or adhesins like PEB1. (3) Evidence is found that *C. fetus* crosses the epithelial barrier via a transcellular route, but (4) a paracellular route accompanied by barrier dysfunction is also likely. (5) Subvasion of epithelial cells prior to *C. fetus* invasion has been observed *in vitro*. (6) Subvasion activity promotes reinvasion from the basolateral side of the epithelial layer, possibly via a CadF-mediated binding to the extracellular matrix protein fibronectin. (7) The S-layer, a crystalline protein structure covering the bacterium, prevents *C. fetus* from phagocytosis by mononuclear cells in the mucosa/submucosa. (8) The T4SS of *C. fetus* is important for invasion of epithelial cells. The Fic effector proteins, possibly secreted by the T4SS, are predicted to alter the host cytoskeleton, promoting further destruction of the epithelial barrier and invasion of deeper layers of the mucosa. (9) Cytolethal distending toxin (Cdt) is a tripartite toxin, which binds to the cell surface via CdtA and CdtC. Delivery of CdtB to the nucleus leads to DNA damage, cell cycle arrest, and ultimately to epithelial cell swelling. The resulting barrier dysfunction promotes dissemination of *C. fetus*. (10) To cause bacteremia, *C. fetus* has to enter the circulation. (11) In the blood stream, the S-layer of *C. fetus* prevents binding of C3b and therefore circumvents killing by the complement system. (12) Antigenic variation of the S-layer occurs within the host, which often leads to relapsing and (13) systemic infection.

Campylobacter virulence envisions that the toxin distends individual cells in the epithelial layer, presumably leading to disruption of the epithelial barrier (i.e. tight junctions) and enabling the bacteria to reach the basolateral cell compartment. The complete *cdt* gene cluster is found in *C. fetus*, and the CdtB protein shows high homologies (~60%) to the *C. jejuni* CdtB [91]. *C. fetus* Cdt shows toxicity against HeLa cells *in vitro* [91]. As is the case with *C. jejuni*, Cdt is not involved in direct invasion of host cells since a *C. fetus cdtB* mutant did not show an altered invasion phenotype in gentamicin protection assays with Caco-2 cells [51]. Moreover, *C. fetus* subspecies harbor homologs of the virulence determinants MviN (MurJ) and the *C*ampylobacter *i*nvasion *a*ntigen (CiaB). The latter has been shown to be secreted via a flagellar Type 3 secretion system in *C. jejuni* and can be detected in the cytoplasm of host cells after infection [78]. In addition, the genomes of both subspecies contain a putative filamentous hemagglutinin of the HecA family whose activity remains to be tested.

S-layer

The best characterized virulence attribute common to both *C. fetus* subspecies so far is the surface (S)-layer, a protein structure covering the bacterial cell surface. Early studies suggested that the S-layer plays a role in resistance to phagocytosis by mononuclear cells. McCoy et al. were the first to purify a surface antigen named antigen [a] [92]. *C. fetus* wild-type strain 23D possesses a specific outer membrane structure that was missing in a *C. fetus* strain lacking antigen [a]. This structure, called the "microcapsule", prevented the cells from phagocytosis by macrophages in the absence of serum, whereas the antigen [a] mutant was efficiently phagocytosed [93]. Moreover, *C. fetus* strains showed a strong resistance to serum killing compared to highly susceptible *C. coli* and *C. jejuni* strains. The observed bactericidal effect was conferred by both complement factors and antibodies [94]. A following study linked the *C. fetus* serum resistance to the presence of high molecular weight surface proteins. The latter were shown to be identical to antigen [a] and represent constituents of the S-layer [95]. Subsequent studies with components of the complement system showed that S-layer encapsulated strains (S^+) did not bind C3b and therefore circumvented complement-mediated killing [96]. To investigate the role of the S-layer *in vivo*, HA/ICR mice were challenged with S^+ and S^- *C. fetus* strains [97]. Intraperitoneal inoculation of S^+ strains compared to S^- strains led to significantly increased mortality. Furthermore, oral inoculation of the S^+ strain led to severe bacteremia, which was not observed in any of the S^- treated mice [97].

The *C. fetus* S-layer is encoded by the chromosomal surface-array protein (sap) locus, about 54 kb in size. At least six *sapA* and *sapB* homologs are prevalent in each (sero-) type A and B strains, respectively, which represent the surface-located constituents of the S-layer called surface layer proteins (SLPs). The predicted proteins show high levels of amino acid identity (~97%) at their amino termini but variation at their carboxyl ends. The conserved regions are responsible for binding of the SLPs to the bacterial LPS. Moreover, the sap locus contains up to 19 additional ORFs including the *sap CDEF* genes responsible for secretion of the sapA/B homologs [5].

To investigate the role of the S-layer in ovine abortion, pregnant ewes were challenged with the *C. fetus* strain 23D and the mutant strain 23B [98] with a deletion in the sapA promoter, the single promoter responsible for SLP expression [99–102]. Although the S-layer was required for establishment and persistence of a chronic infection, it was not the cause of the fetopathogenic effects of *C. fetus* [98]. It is known that *C. fetus* uses complex mechanisms to change expression of the SLP homologs *in vivo*, and therefore evade the immune response by antigenic variation. Several studies explored *C. fetus* antigenic variation during infection of cattle [103–105] and sheep [98, 106], which was found to be conferred by rearrangements within the sap locus leading to alternate expression of the *sapA/B* homologs [104, 105, 107]. The underlying molecular mechanisms utilized for this alternate expression of the *sapA/B* homologs are DNA inversions of a 6.2-kb fragment containing the *sapA* promoter [108, 109]. Inactivation of *recA* led to loss of expression of SLP variants *in vitro*, indicating that RecA is essential for inversion events [110]. However, recombination can also occur independently of *recA* [111], even though with only low efficiency. It was also shown that SLP variation is responsible for the frequently observed relapsing human *C. fetus* infections [112]. Recently, we could show that the *sapA* promoter is subject to temperature-dependent regulation. The promoter activity is reduced at 32 °C compared to 37 °C, suggesting efficient SLP expression only under conditions reflecting the environment in the host (i.e. during the invasive state) wherein SLP expression and antigenic variation are necessary to circumvent immune recognition [68].

Challenges in *C. fetus* research

Much progress has been made in recent years towards understanding the genetics and pathogenesis of *C. fetus*. The sequencing of *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* genomes revealed insights to the evolutionary development of *C. fetus* and provided a genomic basis for detailed investigation of subspecies-specific genes. Importantly, sequencing of different strains within one subspecies that originate from different hosts prompted awareness that the phylogenetic relationships of the subspecies should be reconsidered, and the definition of new subspecies is now subject to discussion.

Probably the most challenging goal in *C. fetus* research will be to establish functions for distinct genes contributing to host–pathogen interactions and niche preferences. Characterization of the *C. fetus* subsp. *venerealis*-specific PAI has brought us an important step further. The island

encodes a functional T4SS for macromolecular transfer of DNA and proteins to other cells. So far, we know that the T4SS contributes to *C. fetus* subsp. *venerealis* invasion in cell culture models of infection and plasmid mobilization between bacteria. Many questions concerning the function of this system remain. Investigation of the two putative *C. fetus* subsp. *venerealis* effector proteins, encoded directly downstream of the *virB/virD4* operon of T4SS genes, is an important next step. Cell-culture-based studies using reporter molecules for secretion activity will be instrumental in visualizing effector translocation to host cell cytoplasm via the T4SS. Detailed characterization of effector protein functions will help us to understand the biology of FIC domain-containing proteins in bacteria and their targeted host cells. In summary, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* are extraordinary model organisms for evaluating the unique contribution of bacterial genes to niche and host adaptations.

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References

1. Allos BM: *Campylobacter jejuni* infections: update on emerging issues and trends. Clin Infect Dis 32, 1201–1206 (2001)
2. Lastovica AJ, Allos BM (2008): Clinical significance of Campylobacter and related species other than *Campylobacter jejuni* and *Campylobacter coli*. In: *Campylobacter*, 3rd edn, eds Nachamkin I, Szymanski CM, Blaser MJ, ASM Press, Washington, DC, pp. 123–149
3. Mead PS, Slutsker L, Dietz V, Mccaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV: Food-related illness and death in the United States. Emerg Infect Dis 5, 607–625 (1999)
4. Blaser MJ: *Campylobacter fetus* – emerging infection and model system for bacterial pathogenesis at mucosal surfaces. Clin Infect Dis 27, 256–258 (1998)
5. Blaser MJ, Newell DG, Thompson SA, Zechner EL (2008): Pathogenesis of *Campylobacter fetus* infections. In: *Campylobacter*, 3rd edn, eds Nachamkin I, Szymanski CM, Blaser MJ, ASM Press, Washington, DC, pp. 401–428
6. Penner JL: The genus Campylobacter: a decade of progress. Clin Microbiol Rev 1, 157–172 (1988)
7. Skirrow MB, Benjamin J: '1001' Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J Hyg (Lond) 85, 427–442 (1980)
8. Logue CM, Sherwood JS, Elijah LM, Olah PA, Dockter MR: The incidence of *Campylobacter* spp. on processed turkey from processing plants in the midwestern United States. J Appl Microbiol 95, 234–241 (2003)
9. Harvey S, Greenwood JR: Isolation of *Campylobacter fetus* from a pet turtle. J Clin Microbiol 21, 260–261 (1985)
10. Rennie RP, Strong D, Taylor DE, Salama SM, Davidson C, Tabor H: *Campylobacter fetus* diarrhea in a Hutterite colony: epidemiological observations and typing of the causative organism. J Clin Microbiol 32, 721–724 (1994)
11. Klein BS, Vergeront JM, Blaser MJ, Edmonds P, Brenner DJ, Janssen D, Davis JP: *Campylobacter* infection associated with raw milk. An outbreak of gastroenteritis due to *Campylobacter jejuni* and thermotolerant *Campylobacter fetus* subsp *fetus*. JAMA 255, 361–364 (1986)
12. Ichiyama S, Hirai S, Minami T, Nishiyama Y, Shimizu S, Shimokata K, Ohta M: *Campylobacter fetus* subspecies *fetus* cellulitis associated with bacteremia in debilitated hosts. Clin Infect Dis 27, 252–255 (1998)
13. Miki K, Maekura R, Hiraga T, Hirotani A, Hashimoto H, Kitada S, Miki M, Yoshimura K, Naka N, Motone M, Fujikawa T, Takashima S, Kitazume R, Kanzaki H, Nakatani S, Watanuki H, Tagusari O, Kobayashi J, Ito M: Infective tricuspid valve endocarditis with pulmonary emboli caused by Campylobacter fetus after tooth extraction. Intern Med 44, 1055–1059 (2005)
14. Skirrow MB, Jones DM, Sutcliffe E, Benjamin J: Campylobacter bacteraemia in England and Wales, 1981–91. Epidemiol Infect 110, 567–573 (1993)
15. Pigrau C, Bartolome R, Almirante B, Planes AM, Gavalda J, Pahissa A: Bacteremia due to Campylobacter species: clinical findings and antimicrobial susceptibility patterns. Clin Infect Dis 25, 1414–1420 (1997)
16. Anstead G, Jorgensen J, Craig F, Blaser M, Patterson T: Thermophilic multidrug-resistant Campylobacter fetus infection with hypersplenism and histiocytic phagocytosis in a patient with acquired immunodeficiency syndrome. Clin Infect Dis 32, 295–296 (2001)
17. Pacanowski J, Lalande V, Lacombe K, Boudraa C, Lesprit P, Legrand P, Trystram D, Kassis N, Arlet G, Mainardi JL, Doucet-Populaire F, Girard PM, Meynard JL: Campylobacter bacteremia: clinical features and factors associated with fatal outcome. Clin Infect Dis 47, 790–796 (2008)
18. Tremblay C, Gaudreau C, Lorange M: Epidemiology and antimicrobial susceptibilities of 111 *Campylobacter fetus* subsp. *fetus* strains isolated in Quebec, Canada, from 1983 to 2000. J Clin Microbiol 41, 463–466 (2003)
19. Gazaigne L, Legrand P, Renaud B, Bourra B, Taillandier E, Brun-Buisson C, Lesprit P: *Campylobacter fetus* bloodstream infection: risk factors and clinical features. Eur J Clin Microbiol Infect Dis 27, 185–189 (2008)
20. Kanj SS, Araj GF, Taher A, Reller LB: *Campylobacter fetus* pericarditis in a patient with beta-thalassemia: case report and review of the literature. Clin Microbiol Infect 7, 510–513 (2001)
21. Dronda F, Garcia-Arata I, Navas E, De Rafael L: Meningitis in adults due to *Campylobacter fetus* subspecies *fetus*. Clin Infect Dis 27, 906–907 (1998)
22. Steinkraus GE, Wright BD: Septic abortion with intact fetal membranes caused by *Campylobacter fetus* subsp. *fetus*. J Clin Microbiol 32, 1608–1609 (1994)
23. Smibert RM: The genus Campylobacter. Annu Rev Microbiol 32, 673–709 (1978)
24. Van Bergen MA, Linnane S, Van Putten JP, Wagenaar JA: Global detection and identification of *Campylobacter fetus* subsp. *venerealis*. Rev Sci Tech 24, 1017–1026 (2005)

25. Perez-Perez GI, Blaser MJ, Bryner JH: Lipopolysaccharide structures of *Campylobacter fetus* are related to heat-stable serogroups. Infect Immun 51, 209–212 (1986)
26. Moran AP, O'malley DT, Kosunen TU, Helander IM: Biochemical characterization of *Campylobacter fetus* lipopolysaccharides. Infect Immun 62, 3922–3929 (1994)
27. Yang LY, Pei ZH, Fujimoto S, Blaser MJ: Reattachment of surface array proteins to *Campylobacter fetus* cells. J Bacteriol 174, 1258–1267 (1992)
28. Tu ZC, Dewhirst FE, Blaser MJ: Evidence that the *Campylobacter fetus sap* locus is an ancient genomic constituent with origins before mammals and reptiles diverged. Infect Immun 69, 2237–2244 (2001)
29. Van Bergen MA, Dingle KE, Maiden MC, Newell DG, Van Der Graaf-Van Bloois L, Van Putten JP, Wagenaar JA: Clonal nature of *Campylobacter fetus* as defined by multilocus sequence typing. J Clin Microbiol 43, 5888–5898 (2005)
30. Tu ZC, Eisner W, Kreiswirth BN, Blaser MJ: Genetic divergence of *Campylobacter fetus* strains of mammal and reptile origins. J Clin Microbiol 43, 3334–3340 (2005)
31. Gilbert MJ, Miller WG, Yee E, Duim B, Wagenaar JA (2011): Whole genome sequencing of *Campylobacter* sp. found in reptiles, *CHRO 2011* poster A420, Vancouver, Canada
32. Gilbert MJ, Miller WG, Yee E, Duim B, Lawson AJ, Blaser MJ, Wagenaar JA (2011): Whole genome sequencing for taxonomic positioning of reptilian *Campylobacter fetus*, *CHRO 2011* poster A421, Vancouver, Canada
33. Van Bergen MA, Van Putten JPM, Dingle KE, Blaser MJ, Wagenaar JA (2008): Isolation, identification, subspecies differentiation, and typing of *Campylobacter fetus*. In: *Campylobacter*, 3rd edn, eds Nachamkin I, Szymanski CM, Blaser MJ, ASM Press, Washington, DC, pp. 213–225
34. Man SM: The clinical importance of emerging *Campylobacter* species. Nat Rev Gastroenterol Hepatol 8, 669–685 (2011)
35. Wang WL, Blaser MJ: Detection of pathogenic Campylobacter species in blood culture systems. J Clin Microbiol 23, 709–714 (1986)
36. Monke HJ, Love BC, Wittum TE, Monke DR, Byrum BA: Effect of transport enrichment medium, transport time, and growth medium on the detection of *Campylobacter fetus* subsp. *venerealis*. J Vet Diagn Invest 14, 35–39 (2002)
37. Lecce JG: Some biochemical characteristics of *Vibrio fetus* and other related Vibrios isolated from animals. J Bacteriol 76, 312–316 (1958)
38. Véron M, Chatelain R: Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. Int J Syst Bacteriol 23, 122–134 (1973)
39. Hum S, Quinn K, Brunner J, On SL: Evaluation of a PCR assay for identification and differentiation of *Campylobacter fetus* subspecies. Aust Vet J 75, 827–831 (1997)
40. Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward DL, Rodgers FG: Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. J Clin Microbiol 40, 4744–4747 (2002)
41. Willoughby K, Nettleton PF, Quirie M, Maley MA, Foster G, Toszeghy M, Newell DG: A multiplex polymerase chain reaction to detect and differentiate *Campylobacter fetus* subspecies *fetus* and *Campylobacter fetus* -species *venerealis*: use on UK isolates of *C. fetus* and other *Campylobacter* spp. J Appl Microbiol 99, 758–766 (2005)
42. Van Bergen MA, Simons G, Van Der Graaf-Van Bloois L, Van Putten JP, Rombout J, Wesley I, Wagenaar JA: Amplified fragment length polymorphism based identification of genetic markers and novel PCR assay for differentiation of *Campylobacter fetus* subspecies. J Med Microbiol 54, 1217–1224 (2005)
43. McMillen L, Fordyce G, Doogan VJ, Lew AE: Comparison of culture and a novel 5' Taq nuclease assay for direct detection of *Campylobacter fetus* subsp. *venerealis* in clinical specimens from cattle. J Clin Microbiol 44, 938–945 (2006)
44. Schulze F, Bagon A, Muller W, Hotzel H: Identification of *Campylobacter fetus* subspecies by phenotypic differentiation and PCR. J Clin Microbiol 44, 2019–2024 (2006)
45. Wagenaar JA, Van Bergen MA, Newell DG, Grogono-Thomas R, Duim B: Comparative study using amplified fragment length polymorphism fingerprinting, PCR genotyping, and phenotyping to differentiate *Campylobacter fetus* strains isolated from animals. J Clin Microbiol 39, 2283–2286 (2001)
46. Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, Van Vliet AH, Whitehead S, Barrell BG: The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature 403, 665–668 (2000)
47. Salama SM, Garcia MM, Taylor DE: Differentiation of the subspecies of *Campylobacter fetus* by genomic sizing. Int J Syst Bacteriol 42, 446–450 (1992)
48. Moolhuijzen PM, Lew-Tabor AE, Wlodek BM, Aguero FG, Comerci DJ, Ugalde RA, Sanchez DO, Appels R, Bellgard M: Genomic analysis of *Campylobacter fetus* subspecies: identification of candidate virulence determinants and diagnostic assay targets. BMC Microbiol 9, 86 (2009)
49. Stynen AP, Lage AP, Moore RJ, Rezende AM, De Resende VD, Ruy Pde C, Daher N, Resende Dde M, De Almeida SS, Soares Sde C, De Abreu VA, Rocha AA, Dos Santos AR, Barbosa EG, Costa DF, Dorella FA, Miyoshi A, De Lima AR, Campos FD, De Sa PG, Lopes TS, Rodrigues RM, Carneiro AR, Leao T, Cerdeira LT, Ramos RT, Silva A, Azevedo V, Ruiz JC: Complete genome sequence of type strain *Campylobacter fetus* subsp. *venerealis* NCTC 10354T. J Bacteriol 193, 5871–5872 (2011)
50. Van Graaf – Van Bloois L, Miller WG, Yee E, Duim B, Wagenaar, JA (2011): Whole genome sequencing of *Campylobacter fetus* subspecies, *CHRO 2011* poster A434, Vancouver, Canada
51. Gorkiewicz G, Kienesberger S, Schober C, Scheicher SR, Gully C, Zechner R, Zechner EL: A genomic island defines subspecies-specific virulence features of the host-adapted pathogen *Campylobacter fetus* subsp. *venerealis*. J Bacteriol 192, 502–517 (2010)
52. Kienesberger S, Gorkiewicz G, Wolinski H, Zechner EL: New molecular microbiology approaches in the study of *Campylobacter fetus*. Microb Biotechnol 4, 8–19 (2011)
53. Kienesberger S, Sprenger H, Wolfgruber S, Thallinger G, Zechner EL, Gorkiewicz G: Comparative genetic analysis of *Campylobacter fetus* subspecies: Model system for bacterial niche specificity and mucosal infection. (2012)
54. Jarvinen N, Maki M, Rabina J, Roos C, Mattila P, Renkonen R: Cloning and expression of *Helicobacter pylori* GDP-l-

fucose synthesizing enzymes (GMD and GMER) in Saccharomyces cerevisiae. Eur J Biochem 268, 6458–6464 (2001)
55. Wu B, Zhang Y, Wang PG: Identification and characterization of GDP-d-mannose 4,6-dehydratase and GDP-l-fucose snthetase in a GDP-l-fucose biosynthetic gene cluster from *Helicobacter pylori*. Biochem Biophys Res Commun 285, 364–371 (2001)
56. McGowan CC, Necheva A, Thompson SA, Cover TL, Blaser MJ: Acid-induced expression of an LPS-associated gene in *Helicobacter pylori*. Mol Microbiol 30, 19–31 (1998)
57. Batchelor RA, Pearson BM, Friis LM, Guerry P, Wells JM: Nucleotide sequences and comparison of two large conjugative plasmids from different *Campylobacter* species. Microbiology 150, 3507–3517 (2004)
58. Friis LM, Pin C, Taylor DE, Pearson BM, Wells JM: A role for the tet(O) plasmid in maintaining *Campylobacter* plasticity. Plasmid 57, 18–28 (2007)
59. Kersulyte D, Velapatino B, Dailide G, Mukhopadhyay AK, Ito Y, Cahuayme L, Parkinson AJ, Gilman RH, Berg DE: Transposable element ISHp608 of *Helicobacter pylori*: nonrandom geographic distribution, functional organization, and insertion specificity. J Bacteriol 184, 992–1002 (2002)
60. Schmidt H, Hensel M: Pathogenicity islands in bacterial pathogenesis. Clin Microbiol Rev 17, 14–56 (2004)
61. Abril C, Brodard I, Perreten V: Two novel antibiotic resistance genes, *tet*(44) and *ant(6)-Ib*, are located within a transferable pathogenicity island in *Campylobacter fetus* subsp. *fetus*. Antimicrob Agents Chemother 54, 3052–3055 (2010)
62. Backert S, Meyer TF: Type IV secretion systems and their effectors in bacterial pathogenesis. Curr Opin Microbiol 9, 207–217 (2006)
63. Fischer W, Haas R, Odenbreit S: Type IV secretion systems in pathogenic bacteria. Int J Med Microbiol 292, 159–168 (2002)
64. Nagai H, Roy CR: Show me the substrates: modulation of host cell function by type IV secretion systems. Cell Microbiol 5, 373–383 (2003)
65. Alvarez-Martinez CE, Christie PJ: Biological diversity of prokaryotic type IV secretion systems. Microbiol Mol Biol Rev 73, 775–808 (2009)
66. Bacon DJ, Alm RA, Hu L, Hickey TE, Ewing CP, Batchelor RA, Trust TJ, Guerry P: DNA sequence and mutational analyses of the pVir plasmid of *Campylobacter jejuni* 81–176. Infect Immun 70, 6242–6250 (2002)
67. Bacon DJ, Alm RA, Burr DH, Hu L, Kopecko DJ, Ewing CP, Trust TJ, Guerry P: Involvement of a plasmid in virulence of *Campylobacter jejuni* 81–176. Infect Immun 68, 4384–4390 (2000)
68. Kienesberger S, Schober Trummler C, Fauster A, Lang S, Sprenger H, Gorkiewicz G, Zechner EL: Interbacterial macromolecular transfer by the *Campylobacter fetus* subsp. *venerealis* type IV secretion system. J Bacteriol 193, 744–758 (2011)
69. Utsumi R, Nakamoto Y, Kawamukai M, Himeno M, Komano T: Involvement of cyclic AMP and its receptor protein in filamentation of an *Escherichia coli fic* mutant. J Bacteriol 151, 807–812 (1982)
70. Worby CA, Mattoo S, Kruger RP, Corbeil LB, Koller A, Mendez JC, Zekarias B, Lazar C, Dixon JE: The fic domain: regulation of cell signaling by adenylylation. Mol Cell 34, 93–103 (2009)
71. Yarbrough ML, Li Y, Kinch LN, Grishin NV, Ball HL, Orth K: AMPylation of Rho GTPases by *Vibrio* VopS disrupts effector binding and downstream signaling. Science 323, 269–272 (2009)
72. Pan X, Luhrmann A, Satoh A, Laskowski-Arce MA, Roy CR: Ankyrin repeat proteins comprise a diverse family of bacterial type IV effectors. Science 320, 1651–1654 (2008)
73. Schmid MC, Scheidegger F, Dehio M, Balmelle-Devaux N, Schulein R, Guye P, Chennakesava CS, Biedermann B, Dehio C: A translocated bacterial protein protects vascular endothelial cells from apoptosis. PLoS Pathog 2, e115 (2006)
74. Zekarias B, Mattoo S, Worby C, Lehmann J, Rosenbusch RF, Corbeil LB: *Histophilus somni* IbpA DR2/Fic in virulence and immunoprotection at the natural host alveolar epithelial barrier. Infect Immun 78, 1850–1858 (2010)
75. Vergunst AC, Schrammeijer B, Den Dulk-Ras A, De Vlaam CM, Regensburg-Tuink TJ, Hooykaas PJ: VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. Science 290, 979–982 (2000)
76. Sory MP, Cornelis GR: Translocation of a hybrid YopE-adenylate cyclase from *Yersinia enterocolitica* into HeLa cells. Mol Microbiol 14, 583–594 (1994)
77. Hu L, Kopecko DJ (2008): Cell Biology of Human Host Cell Entry by *Campylobacter jejuni*. In: *Campylobacter*, 3rd edn, eds Nachamkin I, Szymanski CM, Blaser MJ, ASM Press, Washington, DC, pp. 297–313
78. Larson CL, Christensen JE, Pacheco SA, Minnich SA, Konkel ME (2008): *Campylobacter jejuni* secretes proteins via the flagellar type III secretion system that contribute to host cell invasion and gastroenteritis. In: *Campylobacter* 3rd edn, eds Nachamkin I, Szymanski CM, Blaser MJ, ASM Press, Washington, DC, pp. 315–332
79. Konkel ME, Garvis SG, Tipton SL, Anderson DE Jr., Cieplak W Jr.: Identification and molecular cloning of a gene encoding a fibronectin-binding protein (CadF) from *Campylobacter jejuni*. Mol Microbiol 24, 953–963 (1997)
80. Graham LL: *Campylobacter fetus* adheres to and enters INT 407 cells. Can J Microbiol 48, 995–1007 (2002)
81. Konkel ME, Joens LA: Adhesion to and invasion of HEp-2 cells by *Campylobacter* spp. Infect Immun 57, 2984–2990 (1989)
82. Graham LL, Friel T, Woodman RL: Fibronectin enhances *Campylobacter fetus* interaction with extracellular matrix components and INT 407 cells. Can J Microbiol 54, 37–47 (2008)
83. Baker NT, Graham LL: *Campylobacter fetus* translocation across Caco-2 cell monolayers. Microb Pathog 49, 260–272 (2010)
84. Nielsen HL, Nielsen H, Ejlertsen T, Engberg J, Gunzel D, Zeitz M, Hering NA, Fromm M, Schulzke JD, Bucker R: Oral and fecal *Campylobacter concisus* strains perturb barrier function by apoptosis induction in HT-29/B6 intestinal epithelial cells. PLoS One 6, e23858 (2011)
85. Man SM, Kaakoush NO, Leach ST, Nahidi L, Lu HK, Norman J, Day AS, Zhang L, Mitchell HM: Host attachment, invasion, and stimulation of proinflammatory cytokines by *Campylobacter concisus* and other non-*Campylobacter jejuni* Campylobacter species. J Infect Dis 202, 1855–1865 (2010)

86. Van Alphen LB, Bleumink-Pluym NM, Rochat KD, Van Balkom BW, Wosten MM, Van Putten JP: Active migration into the subcellular space precedes *Campylobacter jejuni* invasion of epithelial cells. Cell Microbiol 10, 53–66 (2008)
87. Pickett CL, Pesci EC, Cottle DL, Russell G, Erdem AN, Zeytin H: Prevalence of cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of *Campylobacter* sp. *cdtB* gene. Infect Immun 64, 2070–2078 (1996)
88. Lara-Tejero M, Galan JE: CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. Infect Immun 69, 4358–4365 (2001)
89. Lee RB, Hassane DC, Cottle DL, Pickett CL: Interactions of *Campylobacter jejuni* cytolethal distending toxin subunits CdtA and CdtC with HeLa cells. Infect Immun 71, 4883–4890 (2003)
90. Lara-Tejero M, Galan JE: A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. Science 290, 354–357 (2000)
91. Asakura M, Samosornsuk W, Taguchi M, Kobayashi K, Misawa N, Kusumoto M, Nishimura K, Matsuhisa A, Yamasaki S: Comparative analysis of cytolethal distending toxin (*cdt*) genes among *Campylobacter jejuni, C. coli* and *C. fetus* strains. Microb Pathog 42, 174–183 (2007)
92. McCoy EC, Doyle D, Wiltberger H, Burda K, Winter AJ: Flagellar ultrastructure and flagella-associated antigens of *Campylobacter fetus*. J Bacteriol 122, 307–315 (1975)
93. McCoy EC, Doyle D, Burda K, Corbeil LB, Winter AJ: Superficial antigens of *Campylobacter* (*Vibrio*) *fetus*: characterization of antiphagocytic component. Infect Immun 11, 517–525 (1975)
94. Blaser MJ, Smith PF, Kohler PF: Susceptibility of *Campylobacter* isolates to the bactericidal activity of human serum. J Infect Dis 151, 227–235 (1985)
95. Blaser MJ, Smith PF, Hopkins JA, Heinzer I, Bryner JH, Wang WL: Pathogenesis of *Campylobacter fetus* infections: serum resistance associated with high-molecular-weight surface proteins. J Infect Dis 155, 696–706 (1987)
96. Blaser MJ, Smith PF, Repine JE, Joiner KA: Pathogenesis of *Campylobacter fetus* infections. Failure of encapsulated *Campylobacter fetus* to bind C3b explains serum and phagocytosis resistance. J Clin Invest 81, 1434–1444 (1988)
97. Pei Z, Blaser MJ: Pathogenesis of *Campylobacter fetus* infections. Role of surface array proteins in virulence in a mouse model. J Clin Invest 85, 1036–1043 (1990)
98. Grogono-Thomas R, Dworkin J, Blaser MJ, Newell DG: Roles of the surface layer proteins of *Campylobacter fetus* subsp. *fetus* in ovine abortion. Infect Immun 68, 1687–1691 (2000)
99. Tummuru MK, Blaser MJ: Characterization of the *Campylobacter fetus sapA* promoter: evidence that the *sapA* promoter is deleted in spontaneous mutant strains. J Bacteriol 174, 5916–5922 (1992)
100. Blaser MJ, Wang E, Tummuru MK, Washburn R, Fujimoto S, Labigne A: High-frequency S-layer protein variation in *Campylobacter fetus* revealed by *sapA* mutagenesis. Mol Microbiol 14, 453–462 (1994)
101. Dworkin J, Blaser MJ: Generation of *Campylobacter fetus* S-layer protein diversity utilizes a single promoter on an invertible DNA segment. Mol Microbiol 19, 1241–1253 (1996)
102. Fujita M, Moriya T, Fujimoto S, Hara N, Amako K: A deletion in the sapA homologue cluster is responsible for the loss of the S-layer in *Campylobacter fetus* strain TK. Arch Microbiol 167, 196–201 (1997)
103. Corbeil LB, Schurig GG, Bier PJ, Winter AJ: Bovine veneral vibriosis: antigenic variation of the bacterium during infection. Infect Immun 11, 240–244 (1975)
104. Wang E, Garcia MM, Blake MS, Pei Z, Blaser MJ: Shift in S-layer protein expression responsible for antigenic variation in *Campylobacter fetus*. J Bacteriol 175, 4979–4984 (1993)
105. Garcia MM, Lutze-Wallace CL, Denes AS, Eaglesome MD, Holst E, Blaser MJ: Protein shift and antigenic variation in the S-layer of *Campylobacter fetus* subsp. *venerealis* during bovine infection accompanied by genomic rearrangement of sapA homologs. J Bacteriol 177, 1976–1980 (1995)
106. Grogono-Thomas R, Blaser MJ, Ahmadi M, Newell DG: Role of S-layer protein antigenic diversity in the immune responses of sheep experimentally challenged with *Campylobacter fetus* subsp. *fetus*. Infect Immun 71, 147–154 (2003)
107. Dubreuil JD, Kostrzynska M, Austin JW, Trust TJ: Antigenic differences among Campylobacter fetus S-layer proteins. J Bacteriol 172, 5035–5043 (1990)
108. Tummuru MK, Blaser MJ: Rearrangement of sapA homologs with conserved and variable regions in *Campylobacter fetus*. Proc Natl Acad Sci U S A 90, 7265–7269 (1993)
109. Dworkin J, Blaser MJ: Nested DNA inversion as a paradigm of programmed gene rearrangement. Proc Natl Acad Sci U S A 94, 985–990 (1997)
110. Dworkin J, Shedd OL, Blaser MJ: Nested DNA inversion of *Campylobacter fetus* S-layer genes is *recA* dependent. J Bacteriol 179, 7523–7529 (1997)
111. Ray KC, Tu ZC, Grogono-Thomas R, Newell DG, Thompson SA, Blaser MJ: *Campylobacter fetus sap* inversion occurs in the absence of RecA function. Infect Immun 68, 5663–5667 (2000)
112. Tu ZC, Gaudreau C, Blaser MJ: Mechanisms underlying *Campylobacter fetus* pathogenesis in humans: surface-layer protein variation in relapsing infections. J Infect Dis 191, 2082–2089 (2005)